bio-protocol Preprint

Not Peer Reviewed
Ask a question online

Protocol of full article

YD Ye Duan

Updated date: Oct 14, 2020

An abbreviated version of this protocol was published in microPublication Biology in Oct 2020

Engineering essential genes with a “jump board” strategy using CRISPR/Cas9

DOI: 10.17912/micropub.biology.000315

Detailed protocol

Protocol for the jump board strategy at *let-7* locus

Ye Duan & Victor Ambros

10.14.2020

Generate the jump board platform strain.

1. Clone precursor-*let-7* and flanking 500 bp sequences into pCR2.1-TOPO vector using lab routine protocols.
2. Mutate precursor-*let-7* sequence in the cloned plasmid to INPP4A (sequences shown in Fig. 1E) using NEB E0554 following the manufacturer’s protocol.
3. PCR amplify to generate dsDNA donor for*let-7-INPP4A (jump board)*using lab routine protocol. We suggest using high-fidelity polymerase for the PCR amplification.
4. Purify the dsDNA donor by routine column purification or ethanol precipitation WITHOUT using glycogen.

(optional) Step 1-4 can be alternatively replaced by using ssDNA donor, if preferred by lab routine.

5. Assembly the injection mix as follow, incubate at room temperature for 10 min for pre annealing, and inject into the gonad of young adults of EG9615.

Name	*Description*	*Stock*	*final*	*µl in*	*20*	*µl* mix
dpy-10	crRNA	160	10	1.25		
let-7-g3	crRNA	200	30	3.00		
tracr	tracrRNA	300	75	5.00		
donor1	dsDNA	89	10	2.25		
H_2O				6.50		
10X duplx.				2.00		

(optional) This recipe has not been exhaustedly optimized. Meanwhile, other protocol with alternative Cas9 (RNP or plasmid), concentrations of components and mix preparation may also be used if preferred by lab routine.

6. After injection, cross the injected P0 with males carrying *mnDp1[umnIs25].*
7. Pick single F1 with co-CRISPR dumpy phenotypes and pharyngeal GFP(*mnDp1).* Let the F1 lay eggs for 1-2 days and then subject the F1 to single worm PCR according lab routine protocol.
8. Genotype the F1 by digesting the PCR products of F1 single worm using EcoRV-HF at 1 unit/µl for 30 min. The F1 animals with INPP4A HDR should hav EcoRV cleavage product (while half of the PCR product, which come from the pairing chromosome and *mnDp1,* should be not be cleaved).
9. Confirm the genotypes by Sanger sequencing.
10. (optional) Back cross the jump board platform strain (VT3742) to remove*dpy-10.* Note that since back crossing is also required for the subsequent edit, it can be skipped here if *dpy-10* was mosaic or heterozygous in F1. We did the back crossing because we plan to use the *ma393* allele as a *let-7(0).*

Generate the engineered *let-7* mutations.

11. Generate dsDNA donor(s) according to Step 1-4. Alternatively, order ssDNA donor(s) if preferred as lab routine.
12. Mix the multiplexed donor with other CRISPR components according to Step 5. Inject into gonads of*mnDp1(+)* young adults of the jump board platform strain.
13. Pick single F1 with pharyngeal GFP and co-CRIPSR dumpy phenotypes.
14. After 1-2 days of egg laying, subject F1 to single worm PCR.
15. Genotype F1 using EcoRV following Step 8. Homozygous HDR and homozygous Indel should have no digestion. We suggest including the un-injected jump board strain as positive control to indicate the EcoRV efficiency.
16. Determine and confirm the homozygous HDR by Sanger sequencing.
17. (Optional) For heterozygous HDR, pick >10*mnDp1(-)* F2 larvae (since *ma393* adults are lethal) from a single F1 and subject to worm PCR, followed by genotyping using EcoRV following above steps. Heterozygous HDR should have un-digested PCR products. Confirm the genotype by Sanger sequencing.
18. If not all the designed mutations are obtained, repeat Step 11-17 with the new injection mix including only the missing HDR donors.
19. Back cross the engineered strains.

Related files